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Remediation potential of early successional pioneer species *Chenopodium album* and *Tripleurospermum inodorum*

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Abstract

Remediation with plants is a technology used to decrease soil or water contamination. In this study we assessed the remediation potential of two weed species (*Chenopodium album* and *Tripleurospermum inodorum*) in a moderately metal-contaminated area. Metal concentrations were studied in roots, stems and leaves, in order to assess correlations in metal concentrations between those in soil and plants. Furthermore, we calculated bioaccumulation factor (BAF), bioconcentration factor (BCF) and translocation factor (TF) values to study the accumulation of metals from soil to plants and translocation within plants. We found correlation in metal concentrations between soil and plants. The metal accumulation potential was low in both species, indicating low BAF and BCF values. In contrast, high TF values were found for Mn, Ni, Sr, Zn, Ba, Fe, Cu and Pb in *C. album*, and for Fe, Mn, Ni, Zn and Sr in *T. inodorum*. Our results demonstrated that the potential of *C. album* and *T. inodorum* might be limited in phytoextraction processes; however, when accumulated, metals are successfully transported to aboveground plant organs. Thus, to achieve the efficient remediation of metal-contaminated soils, removal of the aboveground plant organs is recommended, by which soil disturbance can also be avoided.

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Keywords
bioaccumulation, trace elements, phytoextraction, pollution, translocation

1. Introduction

Unsustainable land use leads to the qualitative and quantitative deterioration of soils, which is an urgent worldwide problem (Mills et al. 2006; Luo et al. 2009; Hoefer et al. 2015). This usually induces the alteration of the macro- and microelemental composition and concentrations of elements in the soil (Ishikawa et al. 2014). Elevated concentrations of potentially toxic metals are known to alter several physical and chemical properties of the growing media and also pose a high risk to organisms (e.g. plants) that are associated with it (Bes et al. 2010; Mahar et al. 2016; Ren et al. 2018a, b). In response, plants could also have elevated metal concentrations in their organs (Del Río-Celestino et al. 2006). The method that utilizes the inherent potential of plants to reduce environmental risk is called phytoremediation (Cunningham and Berti 1993; Flathman and Lanza 1998; Pilon-Smits 2005). Previous studies have revealed that several plants are able to accumulate metals in uncommonly high concentrations. Utilizing this physiological feature, the most successful metal accumulator species are used to extract metals from the contaminated media, thus decreasing the degree of contamination. This type of remediation is called phytoextraction (Dickinson et al. 2009; Zimmer et al. 2009). Moreover, there are other methods of phytoremediation, depending on plant-contaminant interactions (Mahar et al. 2016). Compared to conventional remediation methods, phytoremediation is a low-cost and cost-effective method with a low negative impact on the environment and which establishes long-term green cover in the contaminated area (Vangronsveld et al. 2009).

Field application for phytoextraction purposes usually involves fast growing species characterized by high biomass production (French et al. 2006; Van Nevel et al. 2011; Kacálková et al. 2015; Salam et al. 2019). The metal accumulation potential of plants from natural vegetation was also studied in previous experiments (Brekken and Steinnes 2004; Petřík et al. 2009; Irshad et al. 2015; Bandiera et al. 2016). In natural successions, herbaceous species are reported to play an important role as initial vegetation in terms of phytoremediation, mainly due to their good extraction and degradation rates (Olson et al. 2003). In the later periods of remediation Olson et al. (2003) suggested the use of trees as successfully applicable plants in soils pre-remediated by grasses. Leewis et al. (2013) also demonstrated that natural herbaceous vegetation has good remediation potential and even more significant importance than species used in later remediation phases. Additionally, Malik et al. (2010) highlighted that certain grasses can be used more successfully in phytoremediation processes than trees due to their high growth and biomass production rate. Girdhar et al. (2014) also found that numerous weeds exhibit high levels of inherent metal tolerance. Due to their high colonization ability they are often abundant even in contaminated areas without having harmful effects on the local plant diversity.

Among weeds with these characteristics, information on metal accumulation in Chenopodiaceae species has been widely reported. In this family, annual, stress tolerant *Chenopodium album* (L.) (Lamb's quarters) is one of the most studied species (Hu et al. 2012). Parisien et al. (2015) demonstrated that *C. album* had low bioaccumulation factor (BAF) values regarding soil Cd concentrations. Gupta and Sinha (2008) highlighted that *C. album* had remarkable Fe, Mn, Cd, Cr, Cu, Ni, Pb and Zn accumulation potential, in some cases with bioconcentration factor (BCF) values over 100. Bhargava et al. (2008) also reported that BCF values were high for Fe, Cd and Cu in 13 different accessions of *C. album* and thus recommended application for phytoextraction purposes. Remediation potential can be further increased by adjusting the most optimal row spacing. For *C. quinoa*, Bhargava et al. (2007) found that the highest biomass and thus the highest rate of metal uptake could be reached by sowing at 20–25 cm spacing. In plantations with a low number of individuals manual removal of plants is an efficient harvesting method; in addition, Präger et al. (2018) suggested the use of a combine harvester as a practical method on extensive *C. quinoa* cultivars. Furthermore, when aiming to maximize yield and extraction efficiency by *Chenopodium* species, sowing time is also a major factor to consider (Isobe et al. 2016).

Tripleurospermum inodorum (L.) Sch. Bip. (Scentless mayweed) is an annual, and in some regions overwintering species (Kay 1994). The Cu-binding capacity of *T. inodorum* root exudates was analyzed under hydroponic conditions (Dousset et al. 2001). This species has not yet been used in comprehensive studies assessing metal accumulation potential on contaminated soils. Several studies are available on the related *Matricaria* species, primarily on *Matricaria chamomilla* and *Matricaria recutita* (Armendaríz et al. 2014; Stanojkovic-Sebic et al. 2015). With regards to applicability in phytoextraction, *M. recutita* was found to be a useful metal accumulator species (Radulescu et al. 2013). Unfortunately, the taxonomical classification of species within the *Tripleurospermum* and *Matricaria* genera was controversial and could be used differently by different authors (Applequist 2002). For instance, *Tripleurospermum inodorum* (L.) Sch. Bip. was often referred to as *Matricaria inodora* L. (Samatadze et al. 2014).

The aim of this study was to analyze the metal accumulation and translocation potential of two common, early successional pioneer weeds, *Chenopodium album* and *Tripleurospermum inodorum*, grown in moderately metal-contaminated soils. We explored the difference between the species in terms of their metal concentrations among plant organs and among the differently contaminated parts of the study area. Moreover, accumulation and translocation factors were used to evaluate whether the species, or any of their plant organs (root, stem, shoot (= stem + leaf) and leaf) were capable of accumulating metals in high concentrations. We hypothesized that *C. album* would show excellent remediation potential (high BAF, BCF and TF values). Based on the metal accumulation characteristics of related species, *T. inodorum* was also expected to have good remediation potential.

2. Materials and methods

2.1. Study area

The study area was in the suburban area of Debrecen, Hungary. For the period between 1971 and 2000 the average annual temperature was 10.0 °C, the average annual rainfall was 549 mm and the average annual sunshine duration slightly exceeded 2000 hours (OMSZ n.d.). The 26 ha study area (Lovász-zug, 47°29.0'N; 21°47.3'E) used to function as a series of settling ponds in the communal wastewater treatment process of the city. Secondary biological purification was performed in the area from the 1930s until the 1950s, which was later supplemented by physical treatment. In the initial years of operation, earth deflector walls were formed to facilitate the wastewater stream; thus, the efficiency of purification was greatly increased. From the early 1970s, secondary treatment remained as the only function of the pond system, due to the establishment and continuous development of a modern wastewater treatment plant. The pond system had ceased to operate by the early 2000s (Tőzsér et al. 2018). During the last decades of operation, the study area had been loaded with variable amounts of sewage sludge, contaminated with several macro- and microelements, such as toxic metals.

2.2. Soil sampling and analysis

Soil samples were collected with a 50-mm Dutch soil auger from the three differently contaminated parts of the study area (northern – moderately contaminated part 1; middle – strongly contaminated part 2; southern – moderately contaminated part 3, after Tőzsér et al. (2018); contamination levels were determined by Nagajyoti et al. (2010) and Tóth et al. (2016)) in late September 2015. We selected the soil sampling date to be in alignment with plant sampling near the end of the vegetation period. To spatially arrange the samples, a stratified random design was used. Within each soil core, eight subsamples were collected from increasing depth ranges (0–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80 cm). Samples (N = 216) were put into plastic packages and stored in them at + 4 °C until laboratory work.

To measure the pH of soil solutions, soil samples (5 g) were put into plastic tubes, complemented with 20 ml of deionized water, shaken and left to settle overnight. Then, pH values were determined with a Hach HQ 40d portable multimeter. For the elemental analysis, we homogenized air-dried soil samples (0.2 g with accuracy of 0.005 g) with agate mortar, put into 100-ml glass beakers and dried at 105 °C overnight. Samples were digested in 4 ml 65% (m/m) HNO_3 and 0.5 ml 30% (m/m) H_2O_2 on hot plates until total evaporation of the chemicals. Then, 5 ml of 3×-deionized water was added to the dried samples. Prior to pouring the solution into plastic tubes, we put glass beakers into an ultrasonic water bath to yield the sample residues which adhered to beaker walls. Then, samples were diluted to 10 ml using 1% (m/m)

nitric acid. The following elements were analyzed with MP-AES (Microwave Plasma-Atomic Emission Spectrometry): Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn. These elements are of various levels of environmental concern; we refer to them as "metals" throughout the study, based on Ali and Khan (2018). All concentration values refer to dry weight basis.

2.3. Plant sampling and analysis

Plant individuals were collected from the differently contaminated parts of the study area during early September 2015. We selected the plant sampling date to assess the metal accumulation potential of the species by the end of the vegetation period. Five individuals of *C. album* and *T. inodorum* were collected from a radius of 10 meters around each soil core. We put all samples into plastic packages and stored them at +4 °C until the laboratory process. In the laboratory, individuals were washed and plant organs such as roots, stems and leaves were separated. Each plant organ was air-dried in a paper bag for 24 hours. After this, plant organs were dried at 60°C for 48 hours.

Prior to elemental analyses, 0.2 g of plant samples (with an accuracy of 0.005 g) were homogenized with agate mortar. Then, plant samples were digested in 4 ml 65% (m/m) HNO_3 and 0.5 ml 30% (m/m) H_2O_2. We put the solutions into glass beakers and supplemented them with 3×-deionised water to a quantity of 25 ml. After this, the solutions were put into plastic centrifuge tubes. The following metals were analyzed with MP-AES: Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn.

2.4. Bioaccumulation factor (BAF), bioconcentration factor (BCF) and translocation factor (TF)

We used the bioaccumulation factor (BAF), bioconcentration factor (BCF) and translocation factor (TF) as widely applied indicators in assessing the remediation potential of plant species. For these factors, calculations were made for Al, Fe, Mn, Ba, Cr, Cu, Ni, Pb, Sr and Zn. The bioaccumulation factor refers to the ratio of metal concentration in shoots (C_{shoot}) and metal concentration in soil (C_{soil}) (Li et al. 2007; Rezvani and Zaefarian 2011):

$$BAF = C_{shoot}/C_{soil}$$

The bioconcentration factor refers to the ratio of metal concentration in selected plant organs (also calculated for stem and leaves separately) ($C_{plant\ organ}$) and metal concentration in the soil (C_{soil}) (Ndeda and Manohar 2014):

$$BCF = C_{plant\ organ}/C_{soil}.$$

The translocation factor refers to the ratio of metal concentration in selected aboveground plant organs ($C_{aboveground\ plant\ organ}$) and metal concentration in roots (C_{roots}) (Cui et al. 2007; Mellem et al. 2009; Malik et al. 2010):

$$TF = C_{aboveground\ plant\ organ}/C_{roots}.$$

2.5. Statistical analysis

For the statistical analyses the natural logarithms (ln) of the concentration values were used. Levene's Test was used for analyzing the homogeneities of variances. The Generalized Linear Model (GLM) was used to test significant differences ($p < 0.05$) between the metal concentrations (I) of plants, (II) of plant organs and (III) of parts of the study area with different contamination levels. To study the differences in metal concentration between plant organs, we used a principal component analysis (PCA). To analyze the correlation in metal concentrations between soil samples and plant organs, Pearson's correlation coefficient (r) was calculated and significance was assessed at the 0.05 level.

3. Results

3.1. Metal concentrations in plants among the differently contaminated parts of the study area, plant organs and plant species

The Generalized Linear Model showed that there were significant differences ($p < 0.05$) in Al, Ca, Fe, K, Mg, Mn, Na, Ba and Zn among the differently contaminated parts of the study area. Soil pH did not differ significantly between the three parts of the study area (Table 1). We found significant differences in Al, Ca, Fe, K, Mg, Mn, Na, Ba, Cr, Cu, Ni, Pb, Sr and Zn concentrations among plant organs. Regarding the differences between *C. album* and *T. inodorum*, concentrations of K and Mg were significantly higher in *C. album*, while concentrations of Al, Fe, Mn and Na were significantly higher in *T. inodorum*. Furthermore, mean concentrations of Ba, Cr, Cu, Ni and Pb were higher in *T. inodorum*, while Sr and Zn concentrations were slightly higher in *C. album*; however, these differences were not significant (Suppl. material 1: Tables S1, S2).

3.2. Metal concentrations in *C. album*

There were significant differences in metal concentrations in plant organs of *C. album* among the differently contaminated parts of the study area (Table 2 and Suppl. material 1: Table S3). Metal concentrations were also different among the plant organs of *C. album* based on principal component analysis (PCA) (Fig. 1).

Table 1. Soil pH and metal concentrations in samples from the study area (mean ± SE).

	Part of the study area		
	Northern	**Middle**	**Southern**
pH	7.7 ± 0.1	7.7 ± 0.1	7.8 ± 0.1
Al	5.3 ± 0.2	5.6 ± 0.2	5.7 ± 0.2
Ba	70.1 ± 5.6	65.6 ± 4.1	42.6 ± 1.7
Ca	31.4 ± 2.3	28.2 ± 2.9	19.5 ± 2.2
Cd	0.7 ± 0.1	1.4 ± 0.7	0.7 ± 0.1
Cr	129 ± 21	303 ± 143	63.3 ± 41.8
Cu	35.3 ± 7.2	49.3 ± 13.4	17.6 ± 4.8
Fe	10.6 ± 0.4	11.6 ± 0.6	11.2 ± 0.6
K	1.1 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
Mg	6.2 ± 0.4	5.9 ± 0.3	5.2 ± 0.6
Mn	396 ± 26	380 ± 11	339 ± 20
Na	362 ± 27	363 ± 61	244 ± 24
Ni	23.8 ± 1.6	32.1 ± 5.5	25.8 ± 1.6
Pb	27.4 ± 4.1	44.3 ± 17.7	8.0 ± 4.3
Sr	88.9 ± 7.0	86.4 ± 7.6	55.8 ± 6.3
Zn	153 ± 23	192 ± 45	60.8 ± 15.3

Note: Concentrations of Ba, Cd, Cr, Cu, Mn, Na, Ni, Pb, Sr and Zn are shown in mg kg^{-1}, while concentrations of Al, Ca, Fe, K and Mg are shown in g kg^{-1}.

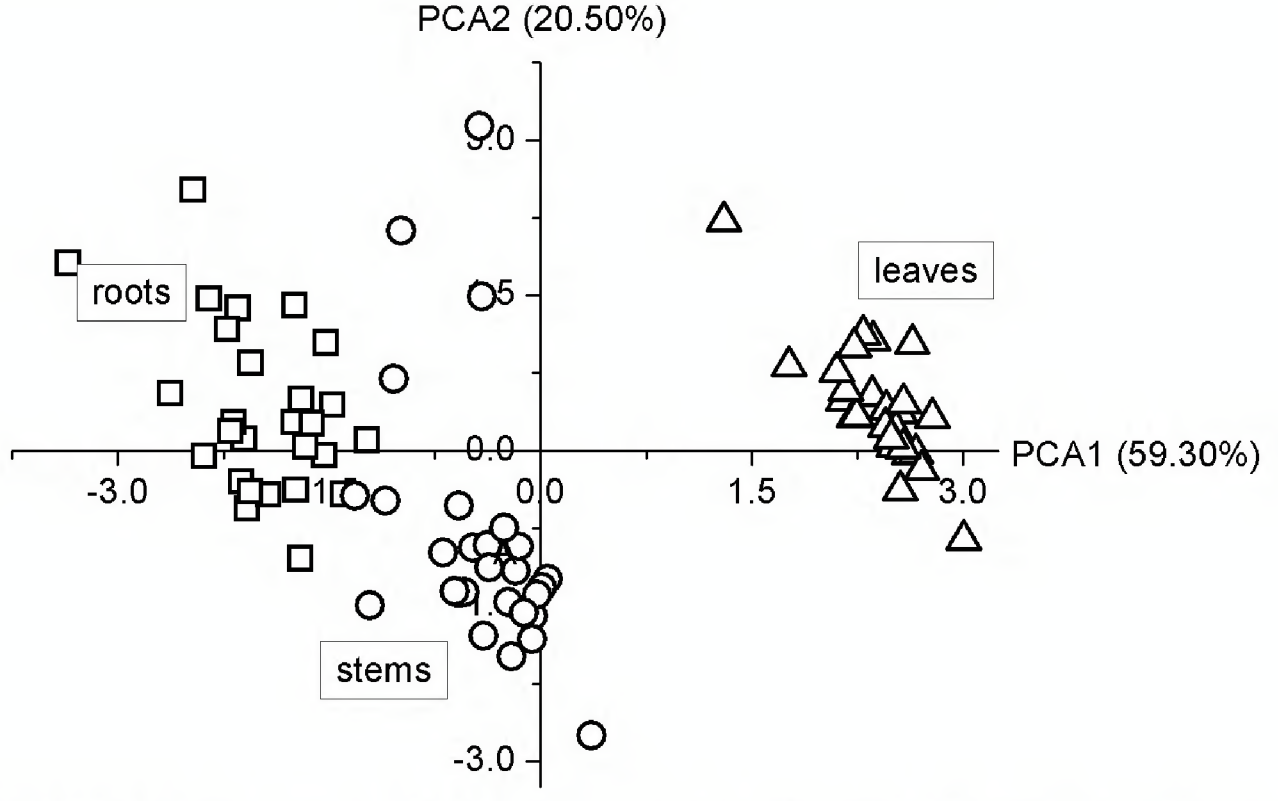

Figure 1. Principal component biplot of metal concentrations (mg kg^{-1}) of *Chenopodium album* in roots, stems and leaves. Notations: square – roots, circle – stems, triangle – leaves.

In the cases of Mg, Mn and Zn, leaves accumulated metals in significantly higher concentrations than roots and stems in all the three parts of the study area. In the cases of Fe and Cu, leaves accumulated significantly higher concentrations of metals than roots and stems only in the strongly contaminated middle part. In the cases of Ca, K and Sr,

Table 2. Metal concentrations in plant organs of *Chenopodium album* among the three parts of the study area (mean ± SE).

	Part of the study area								
	Northern			Middle			Southern		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Al	5.0 ± 0.6	2.0 ± 0.54	3.5 ± 0.6	6.4 ± 2.1	1.1 ± 0.2	2.9 ± 0.3	17.6 ± 3.9	1.2 ± 0.2	5.2 ± 1.8
Ba	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.3 ± 0.02	0.2 ± 0.03	0.1 ± 0.01	0.2 ± 0.02
Ca	0.01 ± 0.01	0.2 ± 0.02	0.9 ± 0.03	0.08 ± 0.01	0.3 ± 0.1	1.0 ± 0.06	0.07 ± 0.01	0.1 ± 0.02	0.9 ± 0.09
Cr	0.1 ± 0.01	0.03 ± 0.01	0.1 ± 0.02	0.1 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.1 ± 0.03	0.02 ± 0.01	0.03 ± 0.01
Cu	0.4 ± 0.02	2.0 ± 1.7	0.5 ± 0.02	0.4 ± 0.01	0.3 ± 0.01	0.5 ± 0.02	0.4 ± 0.01	0.4 ± 0.01	12.2 ± 11.7
Fe	4.9 ± 0.6	5.4 ± 3.3	6.6 ± 0.5	5.9 ± 1.8	2.1 ± 0.2	6.5 ± 0.5	15.0 ± 3.4	2.0 ± 0.2	29.6 ± 23.6
K	0.9 ± 0.04	2.2 ± 0.1	2.9 ± 0.09	1.0 ± 0.03	2.6 ± 0.2	3.1 ± 0.08	1.0 ± 0.05	2.6 ± 0.2	3.0 ± 0.1
Mg	0.1 ± 0.01	0.1 ± 0.01	1.0 ± 0.04	0.1 ± 0.01	0.1 ± 0.03	0.9 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	1.2 ± 0.06
Mn	0.5 ± 0.03	0.5 ± 0.03	1.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.04	1.7 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	4.9 ± 1.5
Na	0.04 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.01	0.1 ± 0.1	0.01 ± 0.0	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
Ni	0.03 ± 0.01	0.02 ± 0.01	0.1 ± 0.1	0.04 ± 0.01	0.1 ± 0.04	0.04 ± 0.01	0.1 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
Pb	0.1 ± 0.01	0.1 ± 0.01	0.03 ± 0.01	0.1 ± 0.04	0.1 ± 0.01	0.03 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Sr	0.9 ± 0.04	1.6 ± 0.1	2.8 ± 0.1	0.8 ± 0.04	1.8 ± 0.2	3.0 ± 0.2	1.0 ± 0.1	1.6 ± 0.1	3.2 ± 0.2
Zn	1.7 ± 0.1	1.5 ± 0.1	5.9 ± 0.3	1.6 ± 0.1	1.4 ± 0.1	5.7 ± 0.5	1.3 ± 0.1	1.1 ± 0.1	2.8 ± 0.2

Note: Concentrations of Al, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn are shown in mg kg^{-1}, while concentrations of Ca, K, Mg and Na are shown in g kg^{-1}.

leaves accumulated the highest concentrations of metals, as well; however, we found significant differences between leaves and stems and also between stems and roots. In the cases of Al and Ba, leaves and roots accumulated metals in significantly higher concentrations than stems. In the cases of Cr and Ni, accumulations in leaves and roots were comparable. Cd concentrations were always below the detection limit; thus, this metal was excluded from further analyses. In the cases of Na and Pb, significant differences were not found among plant organs within the parts of the study area (Suppl. material 1: Table S3).

We also studied metal concentrations in selected plant organs among the differently contaminated parts of the study area. We found higher metal concentrations in the southern part compared to the northern and middle parts of the study area (Table 2 and Suppl. material 1: Table S4). In the southern part of the study area, root Al, Fe, Mn and Ni concentrations were significantly higher than in the northern and middle parts of the area. Root Mg and Ba concentrations were significantly different between the southern and the middle, and between the southern and the northern parts. We observed a significantly higher concentration of Zn in all plant organs in the northern than in the southern part. The middle part did not differ significantly from the other two parts of the study area. Root Ca, K, Na, Cr, Cu and Sr concentrations did not differ significantly among the three parts of the study area.

3.3. Metal concentrations in *T. inodorum*

We also found significant differences in metal concentrations in plant organs of *T. inodorum* among the differently contaminated parts of the study area (Table 3 and Suppl.

Table 3. Metal concentrations in plant organs of *Tripleurospermum inodorum* among the three parts of the study area (mean ± SE).

	Part of the study area								
	Northern			Middle			Southern		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Al	19.5 ± 2.8	7.2 ± 2.0	11.0 ± 1.3	37.4 ± 6.9	7.5 ± 1.3	6.8 ± 0.9	28.3 ± 3.4	6.6 ± 0.8	16.2 ± 5.1
Ba	0.4 ± 0.03	0.2 ± 0.02	0.2 ± 0.01	0.7 ± 0.1	0.3 ± 0.02	0.2 ± 0.01	0.5 ± 0.1	0.3 ± 0.02	0.2 ± 0.02
Ca	0.2 ± 0.02	0.1 ± 0.01	0.8 ± 0.3	0.2 ± 0.02	0.2 ± 0.02	0.6 ± 0.01	0.2 ± 0.03	0.1 ± 0.02	0.6 ± 0.06
Cr	0.2 ± 0.04	0.2 ± 0.04	0.1 ± 0.02	0.4 ± 0.1	0.1 ± 0.02	0.1 ± 0.01	0.2 ± 0.03	0.1 ± 0.01	0.1 ± 0.02
Cu	0.6 ± 0.04	0.3 ± 0.02	0.8 ± 0.1	0.6 ± 0.1	0.3 ± 0.01	0.7 ± 0.03	0.5 ± 0.04	0.3 ± 0.04	22.2 ± 21.5
Fe	17.3 ± 2.5	7.1 ± 1.9	12.2 ± 1.2	32.6 ± 6.0	7.0 ± 1.1	9.2 ± 1.0	22.6 ± 2.6	5.8 ± 0.9	60.4 ± 47.8
K	0.7 ± 0.07	0.6 ± 0.05	1.5 ± 0.1	0.9 ± 0.04	0.6 ± 0.05	1.8 ± 0.05	0.7 ± 0.04	0.7 ± 0.04	1.6 ± 0.1
Mg	0.1 ± 0.01	0.03 ± 0.01	0.3 ± 0.06	0.1 ± 0.01	0.04 ± 0.01	0.2 ± 0.03	0.1 ± 0.01	0.04 ± 0.01	0.2 ± 0.01
Mn	1.5 ± 0.2	1.4 ± 0.2	3.6 ± 0.6	2.5 ± 0.3	3.2 ± 0.6	4.9 ± 0.5	3.1 ± 0.4	3.8 ± 0.7	9.6 ± 1.3
Na	0.2 ± 0.01	0.1 ± 0.01	0.3 ± 0.3	0.1 ± 0.01	0.03 ± 0.01	0.1 ± 0.07	0.2 ± 0.02	0.1 ± 0.01	0.02 ± 0.01
Ni	0.1 ± 0.01	0.1 ± 0.1	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.02
Pb	0.2 ± 0.1	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.1 ± 0.01	0.03 ± 0.01	0.1 ± 0.01
Sr	1.7 ± 0.1	1.2 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.9 ± 0.2
Zn	2.6 ± 0.2	1.9 ± 0.2	2.6 ± 0.4	2.9 ± 0.2	2.7 ± 0.3	2.9 ± 0.3	1.4 ± 0.1	1.4 ± 0.1	1.7 ± 0.2

Note: Concentrations of Al, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn are shown in mg kg-1, while concentrations of Ca, K, Mg and Na are shown in g kg-1.

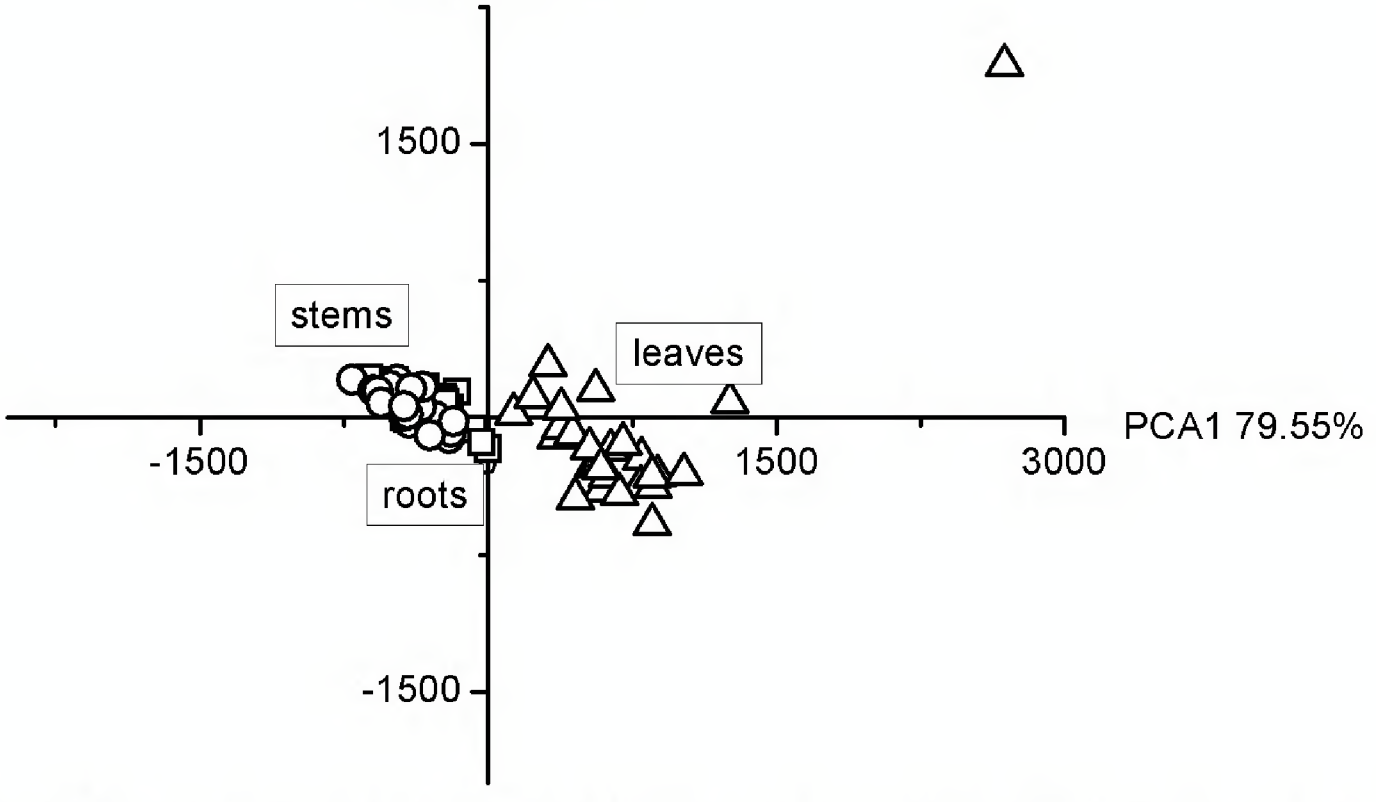

Figure 2. Principal component biplot of metal concentrations (mg kg^{-1}) of *Tripleurospermum inodorum* in roots, stems and leaves. Notations: square – roots, circle – stems, triangle – leaves.

material 1: Table S5). Based on PCA scores, the metals in leaves differed from those in roots and stems of *T. inodorum* (Fig. 2).

Concentrations of Al, Ba, Cr and Pb were significantly the highest in roots, while concentrations of Ca, K, Mg and Mn were the highest in leaves. In the cases of Fe, Na,

Cu, Ni and Sr, the highest concentrations were accumulated in roots and leaves. We found the lowest concentrations of these metals in stems. The concentration of Zn was comparable in all the plant organs, with only negligible differences among them. The concentration of Cd was below the detection limit in all of the cases; thus, this metal was excluded from further analyses.

Generally lower concentrations were found in the northern part compared to the middle and southern parts of the study area (Table 3 and Suppl. material 1: Table S6). In the middle part of the study area, roots accumulated the highest concentrations of Al, K and Ba, whereas leaves accumulated the highest concentrations of Zn. We found the highest Mn concentration in the southern part of the study area regarding all plant organs. Root concentrations of Na and Ni were significantly the highest in the northern and the southern, and in the southern and the middle parts of the study area, respectively. Based on the selected plant organs, Ca, Mg, Cr, Cu, Pb and Sr concentrations did not differ significantly between the differently contaminated parts of the study area.

3.4. Correlations between soil metal concentrations and their accumulation in plants

3.4.1. Correlations between soil metal concentrations and their accumulation in *C. album*

In the northern part of the study area, no significant correlation was found in metal concentrations between the soil and the plant organs of *C. album* (Suppl. material 1: Table S7). In the middle part of the study area, we did not find any negative correlation in the concentrations of Al ($r = -0.700$, $p = 0.036$) and Fe ($r = -0.683$, $p = 0.042$) between the soil and roots and for Pb between the soil and stems ($r = -0.798$, $p = 0.010$). In the southern part of the study area, positive correlations were found in the cases of Cr ($r = 0.733$, $p = 0.025$) and Ni ($r = 0.733$, $p = 0.025$) between the soil and roots. We found negative correlations in the concentrations of Al ($r = -0.783$, $p = 0.013$), Mg ($r = -0.717$, $p = 0.030$) and Ba ($r = -0.731$, $p = 0.025$) between the soil and stems.

3.4.2. Correlations between soil metal concentrations and their accumulation in *T. inodorum*

In the northern part of the study area, Fe concentrations of soil and stems were positively correlated ($r = 0.683$, $p = 0.042$) (Suppl. material 1: Table S8). In the middle part of the study area we found negative correlations in Cu concentrations ($r = -0.883$, $p = 0.002$) between the soil and roots and in Ca concentrations ($r = -0.717$, $p = 0.030$) between the soil and stems. Leaf and soil Sr ($r = 0.733$, $p = 0.025$) concentrations were positively correlated. In the southern part of the study area, soil and root Al concentrations were negatively correlated ($r = -0.667$, $p = 0.050$).

3.5. Metal accumulation and translocation potential in plants

3.5.1. Bioaccumulation factor (BAF), bioconcentration factor (BCF) and translocation factor (TF) values for *C. album*

Bioaccumulation factor (BAF) values were lower than 1 for all metals, which indicates that accumulation was not found in *C. album* (Suppl. material 1: Table S9). Likewise, bioconcentration factor (BCF) values were low in all plant organs (Suppl. material 1: Table S10), which indicated that metal accumulation from soil was also restricted. Some metals had high translocation potential between roots and aboveground plant organs of *C. album* (Suppl. material 1: Table S11). TF values were higher than 1 for Fe, Mn, Ba, Cu, Ni, Pb, Sr and Zn. Very high TF values (> 3) were also demonstrated in leaves in the northern (4.7 for Ni, 3.6 for Zn, 3.4 for Sr), in the middle (3.8 for Sr, 3.7 for Zn, 3.5 for Mn) and in the southern parts of the study area (4.1 for Mn, 3.4 for Sr). In general, compared to roots, accumulation was higher in leaves than in stems. The Pb accumulation in stems was higher than in leaves in all the three parts of the study area, and Ni and Cr in the middle part of the area. We did not observe major differences in TF values among the differently contaminated parts of the study area.

3.5.2. Bioaccumulation factor (BAF), bioconcentration factor (BCF) and translocation factor (TF) values for *T. inodorum*

Corresponding to *C. album*, bioaccumulation factor (BAF) values and bioconcentration factors (BCF) were lower than 1 for *T. inodorum* (Suppl. material 1: Table S9, S10). Translocation factors (TF) were higher than 1 for several metals between roots and aboveground plant organs of *T. inodorum* (Suppl. material 1: Table S11). TF values were higher than 1 for Fe, Mn, Cu, Ni, Sr and Zn. Very high TF values (> 3) were only found in leaves in the southern part of the study area (5.0 for Fe, 3.3 for Mn). Compared to roots, accumulation was slightly higher in leaves than in stems in the middle and southern parts of the study area, while in the cases of Al, Fe, Ba, Cr, Ni and Pb, higher TF values were found in stems than in leaves in the northern part. We did not find significant differences in TF values among the three parts of the study area in the cases of stems, while TF values in leaves were only slightly different among the differently contaminated parts of the study area (southern > middle > northern).

4. Discussion

4.1. Metal concentrations in plants

In the literature discussing environmental pollution, metals are usually classified as (i) trace metals which strongly exceed critical values and (ii) toxic metals which are known

as strong contaminants (Kabata-Pendias and Mukherjee 2007). To determine the level of soil contamination, the threshold values of the Hungarian Governmental Regulation 10/2000. (VI.2.) (2000), Ministry of the Environment Finland (2007) and Tóth et al. (2016) are available for each metal. Cr and Zn concentrations were higher than the threshold values. Cd concentrations were below the detection limit; thus, the metal was not a contaminant in the study area. The Pb and Sr could be potentially risky due to their inherent toxic characteristics; however, their level of contamination was moderate. Based on these, we focused on Cr, Pb and Zn concentrations in soil and plants. Other metals were either not contaminants or their concentrations were too low to be contaminants.

4.1.1. Metal concentrations in *C. album*

We demonstrated that metal concentrations were very low in plant organs of *C. album*. In a previous study Gupta and Sinha (2007) reported higher Cr, Pb and Zn concentrations in plant organs of *C. album* than we did in our study. Similarly to our findings, the authors also demonstrated higher Cr and Pb concentrations in leaves than in roots and stems, but the highest Cu and Zn concentrations were in roots. Studying metal accumulation in *Chenopodium botrys*, Nouri et al. (2009) indicated higher metal concentrations than in our study and found higher Zn concentration in roots compared to shoots. Malik et al. (2010) reported higher Cr, Pb and Zn concentrations in *C. album* than those in this study. They observed higher Cr concentrations in roots than in shoots, but higher Zn concentrations in shoots than in roots. We observed insignificant differences in Cr accumulation among plant organs. As presented in our study, Malik et al. (2010) found similar Pb concentrations in the two plant organs. Nazir et al. (2011) found similar Zn concentrations in soil to concentrations found in this study, but their results showed higher Zn concentrations in the shoots than in the roots of *C. album*. Similarly, we detected significantly higher Zn concentrations in leaves than in roots. To sum up, we found generally lower metal concentrations in organs of *C. album* than reported in the relevant literature, while the distribution of metals within plants was quite varied, as in previous papers.

4.1.2. Metal concentrations in *T. inodorum*

We found that metal concentrations were also very low in plant organs of *T. inodorum*. However, in some cases, we observed significant differences among plant organs even in low concentration ranges. Geneva et al. (2014) found higher Pb and Zn concentrations in roots and shoots of *M. recutita* plants than presented in our paper for *T. inodorum*. A higher Pb concentration was also reported by Lydakis-Simantiris et al. (2012), who also demonstrated that roots accumulated much higher concentrations of the metal than leaves of *M. recutita*, which is a specific characteristic of Pb. In the

cases of Pb and Zn, we did not find significant differences in metal concentrations among plant organs. Low concentrations of Pb could arise from the low mobility and insoluble Pb-complexes in the root zone (Brennan and Shelley 1999). In contrast to our results, the authors found higher Cr concentrations in roots than in stems in each of the five studied species. As a general finding, metal concentrations in *T. inodorum* were lower than presented in the literature for related species.

4.2. Correlations between soil metal concentrations and their accumulation in plants

We found a significant negative correlation in Pb concentrations between the soil and stems of *C. album*. Yoon et al. (2006) also demonstrated a negative correlation in Pb concentrations between the soil and several species of natural vegetation, which was attributed to the inherent low mobility of the metal and basic pH conditions. In contrast to our findings, Jung (2008) found that there were positive correlations in Pb concentrations between the soil and in the plant organs of six crop plant species. Further, by increasing soil concentrations Liang et al. (2016) demonstrated increasing Pb concentrations in plants. We demonstrated significant positive correlations in Cr concentrations between the soil and the roots of *C. album*. In accordance with our findings, Samantaray et al. (2001) demonstrated a significant positive correlation in Cr concentrations between the soil and the roots of four tree species. In a study involving several plant species, including *C. chenopodioides*, Galfati et al. (2011) indicated positive correlations in the concentrations of Cr and Zn between the soil and the accumulation of these metals in plant organs. Despite being quite insoluble, Cr can enter plants easily via ion channels, while essential Zn is readily taken up in concentrations proportional to soil contamination levels.

In the case of *T. inodorum* we found a significant positive correlation in Sr concentrations between the soil and leaves. Lydakis-Simantiris et al. (2012) reported that increasing soil Pb concentrations resulted in elevated Pb concentrations in the roots of *M. recutita* individuals, which was explained by the generally good accumulation potential of the family and by the restricted translocation potential of Pb from roots to aboveground plant organs. In contrast, we did not find significant correlations in Pb concentrations between the soil and plant organs. Greger (1999) found no correlation between soil and *Arrhenatherum pratensis* shoots for Pb and Zn concentrations, mainly due to soil conditions and the formation of complexes that did not favor good bioavailability of metals. Grejtovský et al. (2006) reported that increasing soil Zn concentrations (control, 50, 150 and 300 mg kg^{-1}, respectively) increased Zn concentrations in *M. chamomilla* shoots significantly. Our results indicated that soil metal concentrations did not have any major influence on metal concentrations in plant organs of *C. album* and *T. inodorum*, induced primarily by the relatively high pH values. Nouri et al. (2009) presented similar results, highlighting that plant metal concentrations were basically not influenced by those in the soil, which is the result of the detoxification and exclusion mechanisms developed by certain species. The poor contamination-in-

dicating potential of plant species was also emphasized by Kabata-Pendias and Pendias (1992). According to the Yoon et al. (2006), metal bioavailability, plant-bacteria interactions and several soil parameters could influence metal accumulation jointly. In addition, we found that the multi-contaminated soil of our study area could trigger the uptake of contaminants via interactions between metals (Grejtovský et al. 2006).

4.3. Metal accumulation and translocation potential in plants

4.3.1. Metal accumulation and translocation potential in *C. album*

Bioaccumulation factor (BAF) and bioconcentration factor (BCF) values were simultaneously very low in *C. album*, while translocation factor (TF) values were high in several cases. We found high (> 1) translocation factor (TF) values for Fe, Mn, Ba, Ni, Pb and Sr in the stems and for Fe, Mn, Ba, Cu, Ni, Pb, Sr and Zn in the leaves of *C. album*. In a study on the phytoextraction capacity of *C. album* Gupta and Sinha (2007) reported that leaves accumulated Fe in higher concentrations than roots and stems; thus, the highest TF values appeared for leaves. Similarly to these findings, we found significantly higher Fe concentrations in leaves than in stems, resulting in the highest TF values for leaves. We attributed this to the accumulation-favoring effect of sewage sludge found in deeper soil layers in the study area (Gupta and Sinha 2007). Malik et al. (2010) highlighted that the TF value for Zn was 1.3 for the leaves of *C. album*, which was lower than the values in our study (TF for Zn 2.2–3.7). In addition, Farrag et al. (2013) indicated that the TF values for Mn, Ni and Pb were higher than 1 in the aboveground plant organs of *Chenopodium ambrosioides*, which is similar to our findings. In contrast to our observations they demonstrated that the TF value for Cr was higher than 2. Similarly to what was presented in our study, Zehra et al. (2009) found higher Pb concentrations in aboveground plant organs than in roots (TF > 1) in the cases of three weed species. In contrast, Nazli Alipour et al. (2014) concluded that the accumulation potential of Pb in *C. album* was restricted; the authors demonstrated low (< 0.2) shoot TF values in uncontaminated and also in heavily Pb contaminated soils. It is likely that low mobility and soil organic matter could inhibit Pb accumulation, resulting in low concentrations and mobility in *C. album*. Manan et al. (2015) showed major differences in TF values for Cu, Pb and Zn in two medicinal plants; *Centella asiatica* had TF values higher than 1 for the three metals, which was similar to the results for *C. album* in our study. Furthermore, *Orthosiphon stamineus* had TF values lower than 1 for the three metals. The reported high TF values for Zn in our study could be explained by the fact that this metal is an essential nutrient for plants (Yoon et al. 2006). We detected a high TF value for Mn (4.1) in *C. album*, which is comparable to that presented by Lorestani et al. (2011). However, we found much higher TF values for Cu (5.5) and Fe (6.5) than reported by Lorestani et al. (2011). In comparison with nine other weeds Irshad et al. (2015) reported that *C. album* had similar or higher TF values for Cr and Zn. Furthermore, Gupta and Sinha (2008)

demonstrated that among the five studied weeds *C. album* was the species that showed the highest translocation potential for Zn (TF 3.6) and Ni (2.7), while this species also had a very high translocation potential for Mn (8.6).

4.3.2. Metal accumulation and translocation potential in *T. inodorum*

Bioaccumulation factor (BAF) and bioconcentration factor (BCF) values were also very low for *T. inodorum*, while translocation factor (TF) values deserved attention. We found high (> 1) TF values for Mn, Cu, Ni and Zn in the stems and for Fe, Mn, Cu, Sr and Zn in the leaves of *T. inodorum*. Geneva et al. (2014) presented TF values higher than 1 for Zn (1.3) in *M. recutita* shoots, while lower than 1 for Cu (0.2–0.7) and Pb (0.05–0.30). Compared to these results, TF values for Zn were lower than those shown in our study (TF = 0.8–1.3). In accordance with our results, Lydakis-Simantiris et al. (2012) found low leaf TF values for Ni (0.2–0.3) and Pb (0.1–0.2) in *M. recutita*, indicating low translocation potential for the two metals. Similarly to data presented for stems and leaves in this paper, the average translocation factor value was the highest for Mn (5.3) in a study on Mn, Cr, Ni and Pb accumulations in five weeds conducted by Nwaedozie et al. (2015). They demonstrated higher TF values for Mn than we did in the case of *T. inodorum*; furthermore, they found TF values for Cr, Ni and Pb comparable to those in our paper. Average TF values for Mn (1.1–3.3) were similar to those found in several natural vegetation species by other authors. Studying *Cynodon dactylon*, Kumar et al. (2015) indicated TF values between 1 and 2 for Mn. In a study involving *Cannabis sativa*, *Rumex dentatus* and *Silybum marianum*, Zehra et al. (2009) presented highly varying TF values for Cu, Mn and Zn; however, these values regularly exceeded 1, thereby showing good remediation potential. Based on these findings, we stress that *T. inodorum* showed high levels of Cu-, Mn- and Zn-tolerance and good accumulation of these metals; this was also reported by Liu et al. (2006) for several plant species. In general, comparing the two species studied in this paper, we indicated that *C. album* had a more favorable translocation potential for the studied metals than *T. inodorum*.

4.3.3. Effects of metal interactions on translocation of metals in *C. album* and *T. inodorum*

Translocation factor (TF) values in *C. album* and *T. inodorum* are influenced by several soil parameters. Interactions between metals are typical not only in soils but also in plants. As a common phenomenon, based on their ionic radius, Cd and Zn are in competition for the binding sites located in the transport proteins; thus, they can hinder the accumulation and translocation of each other into and within plants (Hart et al. 1998; Papoyan et al. 2007). This statement was confirmed in our study, since translocation of Zn was much more intensive than that of Cd in both species. Sharma and Dubey (2005) and Pourrut et al. (2011) observed the same mechanism

for both Pb^{2+} and K^{+}, indicating a competing interaction between the two cations for the same K-channel. It is also in accordance with our findings, since we found high TF values for Pb, while that for K remained very low in *C. album*. Furthermore, Fe^{2+}, Mn^{2+} and Zn^{2+} were also found to have identical transporters (Korshunova et al. 1999; Solti et al. 2011), which explains the equally high TF values for these metals presented for *C. album* and *T. inodorum*. It was previously found that Fe cations have the same transportation pathway within plants as Cr^{3+}, resulting in similar concentrations of the two metals (Solti et al. 2011). In contrast, we highlighted the great translocation potential for Fe and low TF values for Cr at the same time. Llugany et al. (2000) reported that Sr^{2+} interferes with K^{+}-metabolism, while there is an inhibiting interaction between Sr^{2+} and Ca^{2+}. We observed high TF values for Sr and low TF values for K, assuming an antagonist effect between the two metals within the two species. Similarly to Llugany et al. (2000), we demonstrated contrasting levels of translocation for Sr and Ca for both species. Based on these we found that ionic radius had a major influence in determining the translocation characteristics observed for *C. album* and *T. inodorum*.

5. Conclusions

The results of this study indicated that both *Chenopodium album* and *Tripleurospermum inodorum* showed low metal accumulation potential in the moderately contaminated study area. Comparing the two species, *T. inodorum* appeared to be a better accumulator of Al, Fe, Mn, Na, Ba, Cr and Ni, while *C. album* was a better accumulator of K, Mg and Sr. Metal concentrations in the two species were generally low. Bioaccumulation factor (BAF) and bioconcentration factor (BCF) values for metals were also low (BAF and BCF < 0.1). In contrast, translocation factor (TF) values were high (> 1) for Fe, Mn, Ba, Cu, Ni, Sr, Pb and Zn in *C. album* and for Fe, Mn, Cu, Sr and Zn in *T. inodorum*. We found that several factors, such as metal interactions and soil characteristics, could influence metal accumulation in plant organs causing a lower accumulation potential of the studied species than reported by the previous studies. Based on the high TF values, aboveground plant organs, especially leaves, could be metal-rich depositories. Summarizing, *C. album* and *T. inodorum* are capable of indicating and accumulating several soil metals, and thus have good potential in the early stages of phytoremediation, assisting the further remediation characterized by woody species.

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Supplementary material I

Tables S1–S11

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Data type: statistical data

Explanation note: **Table S1.** Results of General Linearized Model analysis. **Table S2.** Mean element concentrations of *Chenopodium album* and *Tripleurospermum inodorum*. **Table S3.** Differences in element concentrations among plant organs of *Chenopodium album* within each part of the study area by *p* significance values. **Table S4.** Differences in element concentrations in selected plant organs of *Chenopodium album* among the parts of the study area by *p* significance values. **Table S5.** Differences in element concentrations among plant organs of *Tripleurospermum inodorum* within each part of the study area by *p* significance values. **Table S6.** Differences in element concentrations in selected plant organs of *Tripleurospermum inodorum* among the parts of the study area by *p* significance values. **Table S7.** Correlations in element concentrations between soil and plant organs of *Chenopodium album* in the three parts of the study area. **Table S8.** Correlations in element concentrations between soil and plant organs of *Tripleurospermum inodorum* in the three parts of the study area. **Table S9.** Bioaccumulation factor (BAF) values of *Chenopodium album* and *Tripleurospermum inodorum*. **Table S10.** Bioconcentration factor (BCF) values of *Chenopodium album* and *Tripleurospermum inodorum*. **Table S11.** Translocation factor (TF) values of *Chenopodium album* and *Tripleurospermum inodorum*.

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